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A Novel Member of the Thyroid/Steroid Hormone Receptor Family Is Encoded by the Opposite Strand of the Rat c-*erbA*α Transcriptional Unit

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A cDNA encoding a novel member of the thyroid/steroid hormone receptor superfamily, called Rev-ErbAα, has been isolated from a rat GH_3 cell library. Rev-ErbAα is an ~56-kilodalton protein most similar in structure to the thyroid hormone receptor (c-*erbA*) and the retinoic acid receptor, but it does not bind either thyroid hormone or retinoic acid. The mRNA encoding Rev-ErbAα is present in many tissues and is particularly abundant in skeletal muscle and brown fat. A genomic DNA fragment containing the entire Rev-ErbAα cDNA sequence was isolated and characterized. Remarkably, this DNA fragment also contained a portion of the c-*erbA*α gene. r-*erbA*α-1 and r-*erbA*α-2 are alternative splice products of the c-*erbA*α gene and are members of the receptor superfamily. The genes encoding Rev-ErbAα and r-*erbA*α-2 overlap, with their coding strands oriented opposite one another. A 269-base-pair segment of the bidirectionally transcribed region is exonic in both the Rev-ErbAα and r-*erbA*α-2 genes, resulting in complementary mRNAs. Thus, through alternative splicing and opposite-strand transcription, a single genomic locus codes for three different members of the thyroid/steroid hormone receptor superfamily. Potential implications of this unusual genomic arrangement are discussed.

Cellular homologs of the viral oncogene v-*erbA* (c-*erbA*) encode proteins which bind triiodothyronine (T_3) and together with the steroid hormone and retinoic acid receptors constitute a multigene family of structurally related proteins (9, 14). Similar molecules with unknown ligand specificity have also been isolated (13). Each of these proteins possesses a DNA-binding domain, characterized by a highly conserved cysteine-rich basic region which is thought to interact with specific DNA sequences via zinc fingers, and a ligand-binding domain in its carboxy-terminal region (9, 10).

There are multiple forms of c-*erbA* which have been divided into two subtypes, c-*erbA*α and c-*erbA*β, on the basis of sequence similarities and chromosomal locations (4, 20, 23, 25, 31–33, 38, 40, 41). c-*erbA*α is more closely related to v-*erbA* than is c-*erbA*β. There are at least two c-*erbA*α forms, c-*erbA*α-1 (20, 25, 31, 32, 33, 38, 40) and c-*erbA*α-2 (4, 20, 25, 31, 33). In the rat, the c-*erbA*α-1 and c-*erbA*α-2 proteins are identical for the initial 370 amino acids, after which the sequences diverge (20, 25, 31). The rat c-*erbA*α-1 (referred to hereafter as r-*erbA*α-1) extends another 40 amino acids following the point of divergence from rat c-*erbA*α-2 (referred to as r-*erbA*α-2). The carboxy-terminal (ligand-binding) regions of r-*erbA*α-1, chick c-*erbA*α-1, and c-*erbA*β are highly conserved, and these proteins all bind T_3 with high affinity. In contrast, r-*erbA*α-2 extends 122 amino acids from the point of divergence, and this sequence has no similarity to either r-*erbA*α-1 or c-*erbA*β. r-*erbA*α-2 does not appear to bind thyroid hormone (20, 25, 31), although it specifically binds to a T_3 responsive element in the rat growth hormone gene (25).

Transcription of both strands of the same DNA locus in eucaryotes has been described. Examples of this phenomenon have been reported in a pupal cuticle protein gene in *Drosophila melanogaster* (17), the *D. melanogaster* dopa decarboxylase gene (39), a mouse locus where the function of both 3′-overlapping transcripts is unknown (42), and the rat gonadotropin-releasing hormone gene (1). Transcription from the opposite strand of the c-*myc* gene has also been detected (22, 34).

While investigating the various forms of c-*erbA* represented in a rat GH_3 cell cDNA library, we isolated a cDNA which contained a sequence of 269 nucleotides identical to a sequence present in the r-*erbA*α-2 cDNA we have previously described (25) but which was otherwise unrelated. Surprisingly, the sequence of this cDNA encodes a 56-kilodalton protein when translated in the orientation opposite to that suggested by the region of identity with r-*erbA*α-2. Analysis of the deduced amino acid sequence of this protein, which we call Rev-ErbAα, reveals it to be a novel member of the thyroid/steroid hormone receptor superfamily. The tissue distribution of the Rev-ErbAα mRNA is specific and distinct from those of the r-*erbA*α-1 and r-*erbA*α-2 mRNAs. We have isolated a rat genomic DNA fragment containing sequences common to r-*erbA*α-1 and r-*erbA*α-2, as well as exons encoding the r-*erbA*α-1- and r-*erbA*α-2-specific sequences. Characterization of this genomic fragment confirms that r-*erbA*α-1 and r-*erbA*α-2 are alternative splice products of a single rat gene (25, 32). Furthermore, the complementary strand of this genomic fragment encodes the Rev-ErbAα mRNA. A portion of the r-*erbA*α-2-specific exon is bidirectionally transcribed and encodes a region near the carboxy termini of both the r-*erbA*α-2 and the Rev-ErbAα proteins.

MATERIALS AND METHODS

Isolation and sequencing of Rev-ErbAα cDNA and genomic clones. A cDNA library was constructed in λgt10 from rat

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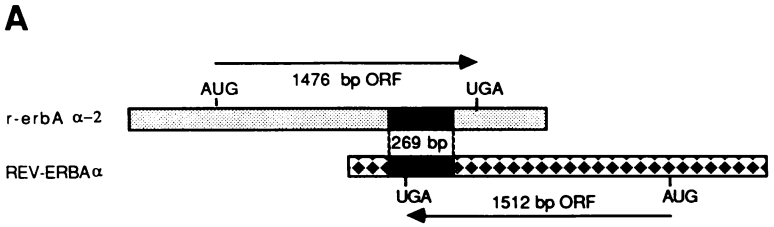

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ATGAGAATATGTCTTTGGCTTTGAAAGAAAGCTGGTGTTATCACCTACATTGGCTCCAGC
GGATCCTCCCCCAGCCGGACCAGCCCAGAGTCCCTCTATAGTGACAGCTCCAATGGCAGC     300
TTCCAGTCCCTGACTCAAGGTTGTCCCACATACTTCCCACCATCACCTACTGGCTCCCTC
ACCCAGGACCCTGCCCGCTCATTTGGCACTGTGCCACCCAGCCTCAGTGATGATAGCTCC     420
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CCCGGAAGTCTACAAGTGGCCATGGAAGACAGCAGCCGAGTGTCCCCCAGCAAGGGCACC     540
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ArgAspAlaValArgPheGlyArgIleProLysArgGluLysGlnArgMetLeuAlaGlu

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MetGlnAsnAlaMetAsnLeuAlaAsnAsnGlnLeuSerSerLeuCysProLeuGluThr

TCACCTGCCCCGCACCCCACCTCAGGCTCCGTGGGCCCCTCACCACCTCCTGCACCAGCC     960
SerProAlaProHisProThrSerGlySerValGlyProSerProProProAlaProAla

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ProThrProLeuValGlyPheSerGlnPheProGlnGlnLeuThrProProArgSerPro

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CAGGGCCCCTCCTCCTACCCTCCTACCTGGCCTTCTGGCCCTGCCCACCACAGCTGCCAC    1320
GlnGlyProSerSerTyrProProThrTrpProSerGlyProAlaHisHisSerCysHis

CAGCCTAACAGCAATGGGCATCGCCTGTGCCCCACCCACGTATACTCGGCCCCAGAAGGC    1380
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AAGGCACCTGCCAACGGTCTACGGCAAGCCAACCACCAAGAATGTTCTGCTGGCATGTCCC    1440
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ATGTTTGACTTCAGCGAGAAGCTCAACTCTCTGGCGCTTACTGAGGAGGAGCTGGGCCTT    1800
MetPheAspPheSerGluLysLeuAsnSerLeuAlaLeuThrGluGluGluLeuGlyLeu

TTCACGGCAGTGGTACTTGTCTCTGCAGACCGCTCGGGAATGCAGAATTTCGGCTTCGGTG  1860
PheThrAlaValValLeuValSerAlaAspArgSerGlyMetGluAsnSerAlaSerVal

GAGCAGCTCCAGCAGACGCTGCTGCGGGCTCTTCGGGCTCTGGTGCTGAAGAACCGGCCC  1920
GluGlnLeuGlnGluThrLeuLeuArgAlaLeuArgAlaLeuValLeuLysAsnArgPro

TCGCAGACTTCCCGCTTCACCAAACTGCTGCTCAAGCTGCCGGACCTGCGGACCCTGAAC  1980
SerGluThrSerArgPheThrLysLeuLeuLeuLysLeuProAspLeuArgThrLeuAsn

AACATGCATTCCGAGAAGCTGCTGTCCTTCCGGGTGGACGCCCAGTGACCGCCCCGGCCG  2040
AsnMetHisSerGluLysLeuLeuSerPheArgValAspAlaGlnEnd

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TCCTGTCTCAAATCCAA                                              2297
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FIG. 1. Structure of the Rev-ErbAα cDNA. (A) Comparison of the Rev-ErbAα and r-*erbA*α-2 cDNAs. The 269 identical nucleotides are shaded black. The orientations of the cDNAs are depicted with the ORFs in opposite orientations. The directions of the ORFs are shown by the arrows, and the putative initiation and termination codons for each ORF are indicated. (B) Nucleotide sequence of the Rev-ErbAα cDNA and deduced amino acid sequence of the Rev-ErbAα protein. The first in-frame termination codon upstream of the putative initiator methionine is underlined. The 269 nucleotides (1826 to 2094) identical to r-*erbA*α-2 are shown in boldface type. The upper and lower boxed portions of the sequence correspond to the putative DNA- and ligand-binding domains analyzed in greater detail in Fig. 2A and B, respectively.

GH_3 cell poly(A)$^+$ RNA (24) and screened with a ^{32}P-labeled (11) fragment (base pairs [bp] 871 to 1867) of the r-*erbA*α-2 cDNA that we have previously described (25). One hybridizing cDNA clone, referred to as Rev-ErbAα, was subcloned into M13 and Bluescript, and both strands were sequenced by using the dideoxy chain termination method (37). Three Rev-ErbAα cDNAs of smaller size were obtained from a rat brain λgt11 cDNA library (Clontech, Palo Alto, Calif.).

A Charon 4A rat genomic library (partial *Hae*III; kindly provided by J. Bonner) was screened with the Rev-ErbAα cDNA. Three hybridizing clones were obtained, the longest of which also hybridized to an r-*erbA*α-specific probe (25). This DNA fragment was subcloned into Bluescript, sequenced by using the dideoxy nucleotide chain termination method (37), and mapped by standard techniques (27).

In vitro transcription and translation. The original GH_3 cell-derived Rev-ErbAα cDNA and a truncated Rev-ErbAα cDNA (digested with *Bam*HI to remove 260 untranslated nucleotides at the extreme 5′ end) were subcloned into Bluescript and transcribed into RNA by using T7 RNA polymerase. The RNA was translated in reticulocyte lysates in the presence of [^{35}S]methionine as previously described (25). The truncated cDNA yielded more high-molecular-weight protein than the original cDNA and was used in the experiment shown. The labeled products of the in vitro translation reactions were analyzed by electrophoresis on sodium dodecyl sulfate-10% polyacrylamide gels alongside ^{14}C-labeled molecular weight markers.

RNA preparation and analysis. Fragments of the cDNAs were subcloned into Bluescript in both orientations, and these constructs were used to generate cRNA probes. The fragments subcloned were from the r-*erbA*α-2 cDNA (bp 871 to 1867) (25), including the sequence shared by the Rev-ErbAα and r-*erbA*α-2 cDNAs (generating α-2 sense and α-2 antisense cRNA probes, respectively), and from the Rev-ErbAα cDNA (bp 1 to 1845; Fig. 1B) which includes 20 nucleotides common to r-*erbA*α-2 but did not hybridize to r-*erbA*α-2 mRNA or cDNA in either orientation, presumably because of stringent hybridization conditions. RNA was prepared from cells and tissues by the guanidinium thiocyanate extraction method (7). GH_3 cells were cultured and incubated in the presence or absence of 10 nM T_3 as

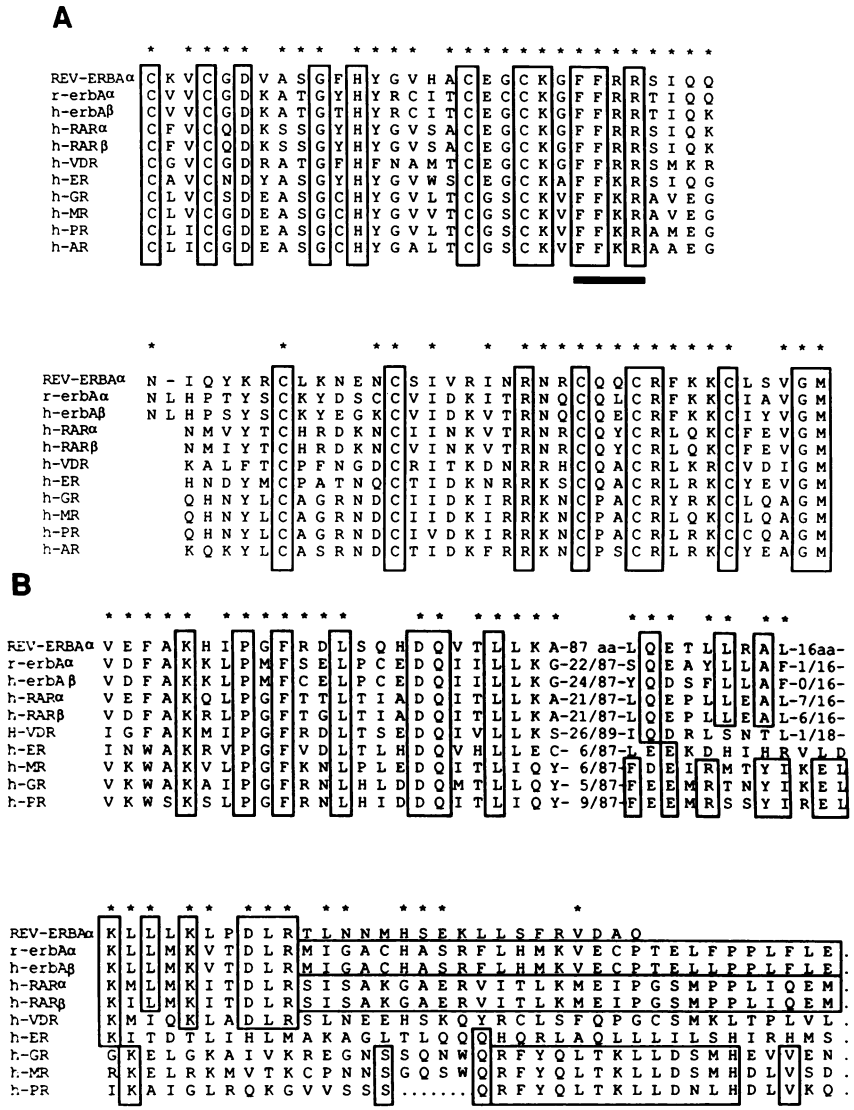


FIG. 2. Comparison of the deduced Rev-ErbAα amino acid sequence with members of the thyroid/steroid hormone receptor superfamily. The one-letter amino acid code is used. The regions of the Rev-ErbAα protein correspond to the boxed areas in Fig. 1B. Asterisks indicate residues identical between Rev-ErbAα and c-*erbA*, retinoic acid receptor, or vitamin D receptor. Abbreviations: r-erbAα, rat c-*erbA*α-1 (40); h-erbAβ, human c-*erbA*β (41); h-RARα, human retinoic acid receptor α (12, 35); h-RARβ, human retinoic acid receptor β (8); h-ER, human estrogen receptor (15, 16); h-VDR, human vitamin D receptor (3, 28); h-GR, human glucocorticoid receptor (18); h-MR, human mineralocorticoid receptor (2); h-PR, human progesterone receptor (30); h-AR, human androgen receptor (6, 26). (A) Comparison of the putative DNA-binding regions. The Rev-ErbAα sequence begins with amino acid 25 in the deduced amino acid sequence corresponding to the upper boxed region in Fig. 1B. Upper and lower parts of this figure are continuous. Boxed amino acids indicate residues conserved in all members of the superfamily. The underlined amino acids F-F-R/K-R present between the two cysteine-rich sequences are discussed in the text. (B) Comparison of the putative ligand-binding regions. The Rev-ErbAα sequence begins with amino acid 345 in the deduced amino acid sequence shown in Fig. 1B and corresponds to the lower boxed region in Fig. 1B. Upper and lower parts of this figure are continuous. Boxed amino acids in the first 24 residues shown indicate amino acids conserved among all members of the family. Eighty-seven- and sixteen-amino-acid regions of lesser homology between Rev-ErbAα and other members of the family are indicated in the upper half of the figure by the fraction of amino acids identical without introducing gaps in the sequences (except in the h-VDR and h-PR sequences). Other boxes indicate conserved residues among some members of the family, highlighting the similarities among Rev-ErbAα, c-*erbA*α and c-*erbA*β, and h-RARα and h-RARβ, as well as the similarities among h-GR, h-PR, and h-MR. The decreasing homologies at the extreme carboxy termini of the proteins are discussed in the text.

previously described (24). Ten μg of total RNA was applied to each lane of 1% agarose-formaldehyde gels, and Northern (RNA) blot analyses were performed by standard techniques (27). Blots were hybridized at 65°C in the presence of 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5% sodium dodecyl sulfate and washed at 65 to 75°C in the presence of 0.2× SSC–0.1% sodium dodecyl sulfate.

Southern analysis. Rat genomic DNA was obtained from Clontech. Restriction digests were performed by using standard procedures. Ten μg of rat genomic DNA was digested with either *Eco*RI, *Pst*I, *Bam*HI, or *Hin*dIII and subjected to

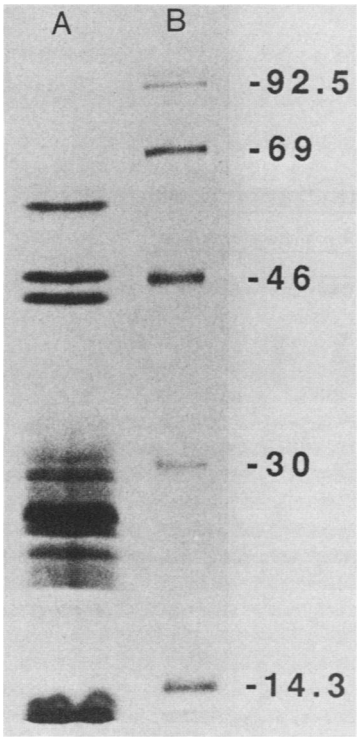


FIG. 3. In vitro translation of RNA transcribed from the Rev-ErbAα cDNA. The Rev-ErbAα protein products shown in lane A were subjected to electrophoresis. The three largest proteins seen are approximately 56, 46, and 44 kilodaltons. Other lower-molecular-weight species may represent degradation products of the larger proteins or translation from alternative, downstream initiation sites. Radiolabeled standard proteins shown in lane B were subjected to electrophoresis, with molecular mass indicated in kilodaltons.

electrophoresis in 0.8% agarose and Southern analysis by using standard techniques (27). Blots were hybridized at 42°C in 2× SSC–50% formamide and washed at 50°C in 2× SSC–0.1% sodium dodecyl sulfate.

RESULTS

Isolation of the Rev-ErbAα cDNA. A λgt10 rat GH_3 cell cDNA library was screened with restriction DNA fragments corresponding to the 3′ end of the r-*erbA*α-2 cDNA (25). The insert in one cDNA, which we call Rev-ErbAα, was 2.3 kilobases (kb) in length and contained a sequence of 269 nucleotides identical to a region of r-*erbA*α-2 but was otherwise completely dissimilar (Fig. 1A). No long open reading frame (ORF) was found in the nucleotide sequence of this cDNA when it was analyzed in the 5′ to 3′ orientation with respect to the sequence identical to the r-*erbA*α-2 cDNA. However, when the Rev-ErbAα cDNA was translated in the opposite orientation with respect to the r-*erbA*α-2-identical nucleotides, a 1,512-nucleotide ORF encoding a 504-amino-acid protein was deduced (Fig. 1B).

Rev-ErbAα is a thyroid/steroid hormone receptor-like protein. The deduced amino acid sequence of the Rev-ErbAα protein contains regions which are remarkably similar to the conserved sequences of the proteins which constitute the thyroid/steroid hormone receptor superfamily, including r-*erbA*α-1 and r-*erbA*α-2. As with the other members of this gene family, the degree of similarity among the putative DNA-binding domains (Fig. 2A) is greater than among the ligand-binding domains (Fig. 2B). The deduced amino acid sequence of the Rev-ErbAα protein is most similar to those of the c-*erbA* proteins and the retinoic acid receptors and is as much like the other members of the receptor superfamily as are the thyroid hormone and retinoic acid receptors. The amino acid similarities among all members of the superfamily in the proximal portion of the ligand-binding regions are highlighted in Fig. 2B. As the sequences progress towards their carboxy termini, the Rev-ErbAα/c-*erbA*/retinoic acid receptor/vitamin D receptor sequences diverge from the glucocorticoid/mineralocorticoid/progesterone receptor sequences (note the boxed areas in Fig. 2B). Towards the extreme carboxy termini which presumably are most involved with ligand specificities (9, 14), the similarities between Rev-ErbAα, c-*erbA*, and retinoic acid and vitamin D receptors lessen considerably.

The Rev-ErbAα cDNA was subcloned into Bluescript and transcribed into RNA. This RNA was translated in reticulocyte lysates and produced three major protein species of 56, 46, and 44 kilodaltons as shown in Fig. 3. The size of the largest-molecular-weight protein is similar to that deduced from the Rev-ErbAα amino acid sequence. The in vitro synthesized Rev-ErbAα protein did not specifically bind T_3, reverse T_3, retinoic acid, or cholesterol (data not shown).

Rev-ErbAα mRNA is complementary to r-*erbA*α-2 mRNA. To prove that the Rev-ErbAα and r-*erbA*α-2 cDNAs are derived from mRNAs containing complementary sequences, we performed Northern analyses by using single-stranded cRNA probes. The correct orientations of the cRNA probes were confirmed by dot blot hybridization with single-stranded M13 DNA preparations which had been used to sequence the Rev-ErbAα cDNA (data not shown). Figure 4B shows the expression of Rev-ErbAα mRNA in a variety of rat tissues with a cRNA probe which was antisense with respect to the strand of the Rev-ErbAα cDNA which encoded the long ORF (Fig. 4A). A single mRNA, of approximately 3.0 kb, was detected in all tissues examined. The Rev-ErbAα mRNA was most abundant in skeletal muscle and brown fat. No 2.6-kb band corresponding to r-*erbA*α-2 was noted. The sense probe with respect to the ORF did not hybridize significantly to RNA from these tissues (data not shown). Figures 4C and 4D show that RNA probes containing the sequence common to r-*erbA*α-2 and Rev-ErbAα hybridized to mRNA from a number of rat tissues. Figure 4C shows that the cRNA probe which is antisense with respect to the r-*erbA*α-2 mRNA hybridized to a 2.6-kb mRNA with a tissue distribution similar to that which we have previously described for r-*erbA*α-2 (25). The level of this mRNA in GH_3 cells decreased after 24 h of treatment with 10 nM T_3 as previously reported (24). In contrast, the cRNA probe which is sense with respect to the r-*erbA*α-2 mRNA hybridized to a 3.0-kb mRNA which presumably is the Rev-ErbAα mRNA. The tissue distribution of this 3.0-kb mRNA was similar to that noted for the Rev-ErbAα mRNA (Fig. 4B). Moreover, unlike those of r-*erbA*α-2, levels of the 3.0-kb mRNA in GH_3 cells did not vary as a function of T_3 treatment. When a double-stranded cDNA probe is used in similar experiments, a broadly hybridizing band which occasionally can be resolved into a doublet is seen (data not shown). Thus, although two distinct mRNAs contain the nucleotide sequence shared between the r-*erbA*α-2 and Rev-ErbAα cDNAs, this nucleotide sequence is present in opposite orientations in the two mRNAs.

Rev-ErbAα, r-*erbA*α-1, and r-*erbA*α-2 are encoded at a single genomic locus. We have previously reported that

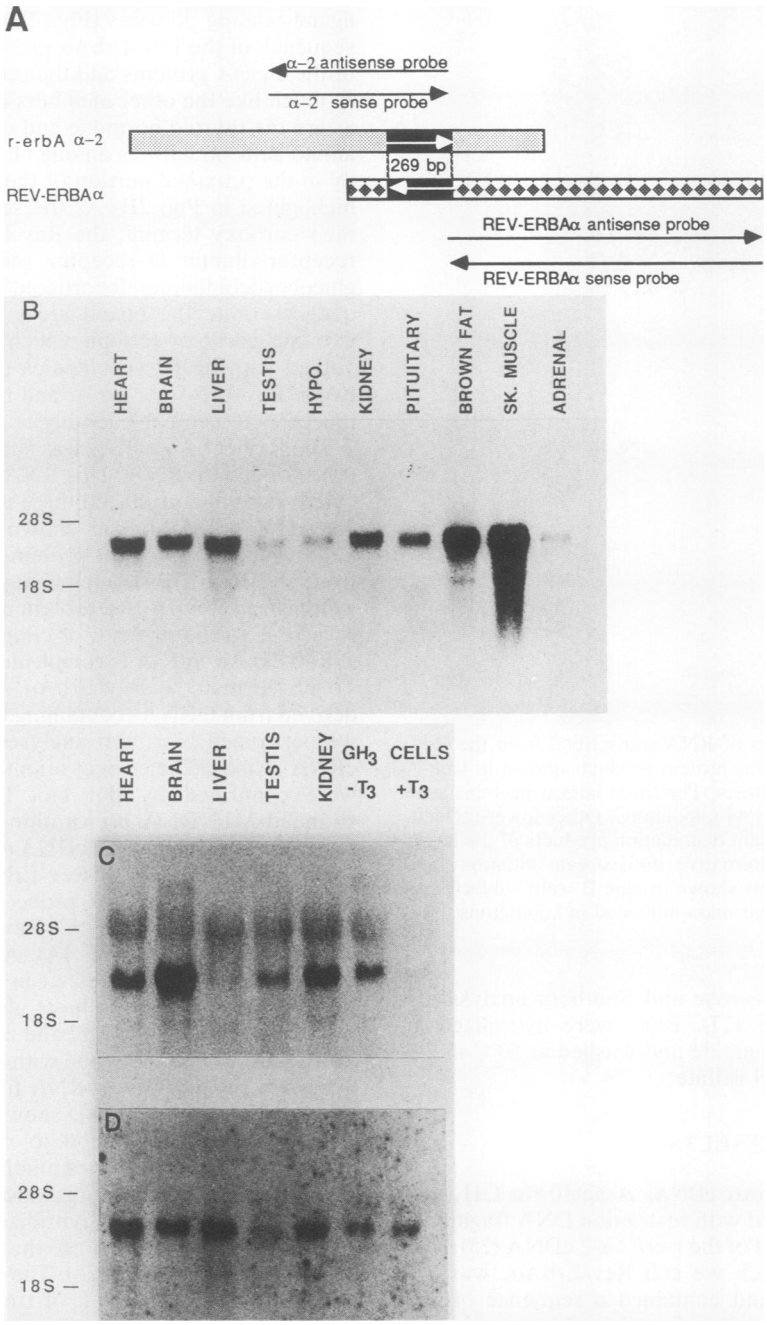


FIG. 4. Northern analysis of Rev-ErbAα mRNA in rat tissues. (A) cRNA probes used for Northern analysis. The structures of the Rev-ErbAα and r-*erbA*α-2 cDNAs are shown as in Fig. 1A. The Rev-ErbAα-specific cRNA probes are indicated as Rev-ErbAα sense and Rev-ErbAα antisense probes. The Rev-ErbAα antisense probe and the r-*erbA*α-2 sense probe are complementary to the ORF-encoding strand in the Rev-ErbAα cDNA. The r-*erbA*α-2 antisense probe is complementary to the ORF-encoding strand in the r-*erbA*α-2 cDNA. (B) Hybridization of the Rev-ErbAα antisense probe to 10 μg of total RNA from a variety of rat tissues. Abbreviations: HYPO, hypothalamus; SK. MUSCLE, skeletal muscle. (C) Hybridization of the r-*erbA*α-2 antisense probe to 10 μg of total RNA from a variety of rat tissues and from GH_3 cells incubated in the presence or absence of T_3. The hybridization in the region of 28S rRNA seen with this probe probably represents a combination of nonspecific binding to the 28S rRNA as well as comigration of r-*erbA*α-2 mRNA with 28S rRNA, since the signal is somewhat proportional to that of the 2.6-kb r-*erbA*α-2 mRNA and ethidium bromide staining confirmed that approximately equal amounts of RNA were loaded in each lane. (D) Hybridization of the r-*erbA*α-2 sense probe to 10 μg of total RNA from a variety of rat tissues and from GH_3 cells incubated in the presence or absence of T_3.

Southern analyses suggest that r-*erbA*α-1 and r-*erbA*α-2 are encoded by a single rat gene (25). Figure 5 shows Southern analyses of rat genomic DNA digested with four different restriction enzymes and probed either with a cDNA fragment common to r-*erbA*α-2 and Rev-ErbAα (probe A) or with a Rev-ErbAα-specific fragment (probe B). With probe A, single hybridizing restriction fragments are seen in each lane of the Southern blot, confirming that the sequence

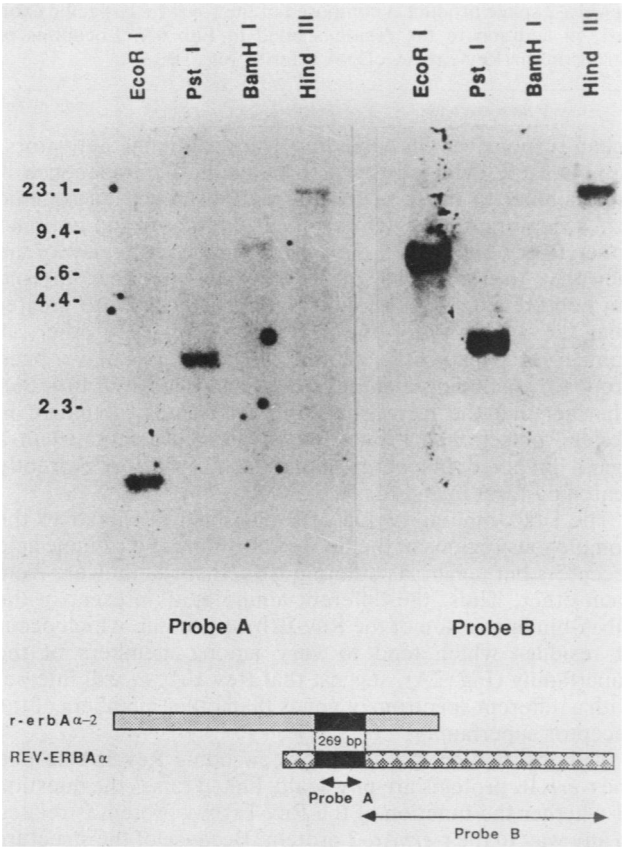


FIG. 5. Southern blot hybridization of rat genomic DNA, with common Rev-ErbAα/r-*erbA*α-2 (probe A) or Rev-ErbAα-specific (probe B) probes. Probe A corresponds to bp 1845 to 2065 (an *Eco*RI-*Rsa*I fragment) in the Rev-ErbAα cDNA (Fig. 1B) and bp 1653 to 1873 in the r-*erbA*α-2 cDNA (25). Probe B corresponds to bp 1 to 1845 in the Rev-ErbAα cDNA (Fig. 1B). On longer exposures, faint additional hybridizing restriction fragments were seen in the *Pst*I (~1.9 kb) and *Bam*HI (~1 kb) digests. Similar fragments were also noted in Southern analyses of the genomic fragment described in Fig. 6 and the text (data not shown). Sizes of marker DNA fragments are in kb.

common to r-*erbA*α-2 and Rev-ErbAα is present as a single copy in the rat genome. Probes A and B hybridized to restriction fragments of identical size in the *Bam*HI and *Hin*dIII digests but not in the *Eco*RI and *Pst*I digests, consistent with the restriction map of the genomic fragment which will be described subsequently (*Eco*RI, Fig. 6A; *Pst*I, data not shown). Although probe B contains a *Hin*dIII restriction site, only one fragment hybridizing to the Rev-ErbAα probe was detected in the *Hin*dIII digest of genomic DNA. This is because of the presence of a *Hin*dIII site at the junction of two exons (exon 2 and exon 3; Fig. 6A and C) in the gene structure.

A rat genomic library was screened with the two probes used for Southern analyses, and two clones were obtained which hybridized to both probes. One of these clones also hybridized to a r-*erbA*α-1-specific probe and to a probe detecting sequences common to r-*erbA*α-1 and r-*erbA*α-2; this clone was selected for extensive DNA sequence and restriction enzyme analyses (Fig. 6).

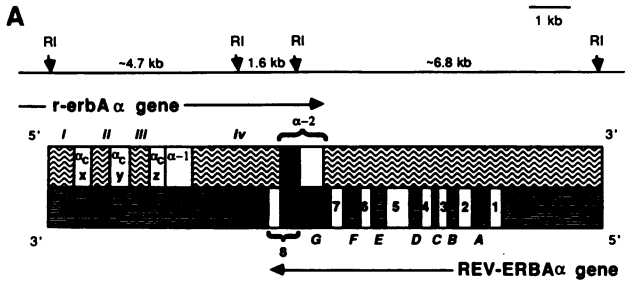
Organization of the 3′ end of the r-*erbA*α gene. A portion of the sequence common to the r-*erbA*α-1 and r-*erbA*α-2 cDNAs is contained in an ~4.7-kb *Eco*RI fragment. The 5′-most exon/intron boundaries that we have identified (introns *i* and *ii*; Fig. 6) occur at the exact same position as in the chick c-*erbA*α gene (where these are the most 3′ of the introns that have been studied) (43). Three exons (exons x, y, and z; Fig. 6) encoding amino acid sequences common to r-*erbA*α-1 and r-*erbA*α-2 were characterized. The 3′-most of these exons (exon z) consists of sequences common to the r-*erbA*α-1 and r-*erbA*α-2 mRNAs, adjacent (without an intron) to the r-*erbA*α-1-specific sequence (Fig. 6A). This exon contains all of the r-*erbA*α-1-specific sequence found in the cDNA clones, which are approximately 2-kb in size (25, 32, 40). The exonic sequence may continue further, since the r-*erbA*α-1 mRNA is 5.0-kb in size (25). There are potential polyadenylation signals ~2.7 kb (AATAAA) and ~2.9 kb (AATAAG) downstream of the end of the r-*erbA*α-1 cDNA-encoding region. Of note, the first two r-*erbA*α-1 specific nucleotides are GT, which likely serves as the splice donor when the r-*erbA*α-2 mRNA is produced. The r-*erbA*α-2-specific exon is located ~3,700 nucleotides downstream of the r-*erbA*α-common and r-*erbA*α-1 junction (Fig. 6A and C). Since the r-*erbA*α-2 cDNA sequence which we have reported is not polyadenylated, it is unclear exactly how far 3′ the r-*erbA*α-2-specific exon extends in the gene; however, the 2,461-bp cDNA is close in size to the 2.6-kb mRNA. There is a potential polyadenylation signal, CATAAA, at the 3′ end of this cDNA (25).

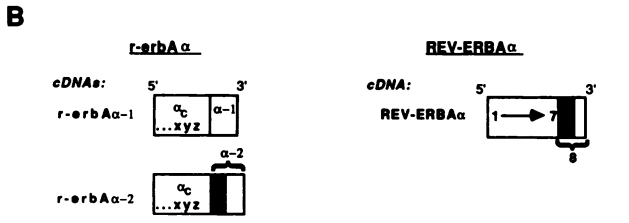
Rev-ErbAα is encoded by the opposite strand of the r-*erbA*α-2 transcriptional unit. The entire Rev-ErbAα cDNA sequence is also contained in the genomic fragment described above. Since the Rev-ErbAα mRNA is 3.0 kb and the cDNA is 2.3 kb in size, there may be exons containing untranslated regions which we have not yet isolated. The Rev-ErbAα cDNA is encoded by eight exons. All eight exons and the seven introns were sequenced; their characteristics are summarized in Fig. 6C. The exon/intron junctions all have appropriate splice donor/acceptor sites (data not shown). A portion of exon 8 in the Rev-ErbAα gene is an intron in the r-*erbA*α-2 gene which is encoded on the opposite strand. The two zinc fingers in the DNA-binding domain of the putative Rev-ErbAα protein (Fig. 2A) are encoded by separate exons (exons 3 and 4). The site of the intron separating the coding sequences for the two domains is just 5′ to the exon encoding the F-F-R-R sequence (underlined in Fig. 2A) characteristic of the thyroid hormone, retinoic acid, and vitamin D receptors. This is the exact site of the analogous intron in the chick c-*erbA*α gene (43). This intron position contrasts, however, with the human mineralocorticoid receptor (2) and the chick progesterone receptor (19) genes, which have introns separating the two zinc finger-encoding exons after the related F-F-K-R sequence. This intron is relatively small in the Rev-ErbAα (93 bp) and chick c-*erbA* (85 bp) genes, but it is over 10 kb in size in the chick progesterone receptor gene (19).

The 269-nucleotide region common to the r-*erbA*α-2 and Rev-ErbAα cDNAs derives from the same genomic segment, which is bidirectionally transcribed (Fig. 6A). A portion of the r-*erbA*α-2-specific exon is partly intronic with respect to generation of the Rev-ErbAα mRNA. Analogously, exon 8 in the Rev-ErbAα gene is partly intronic in the r-*erbA*α-2 gene. In both instances, splice donor (GT) and acceptor (AG) sequences are present on the appropriate DNA strands.

DISCUSSION

The structure of Rev-ErbAα is remarkably similar to the structure of a number of hormone receptors which are





C

Intron/Exon Boundaries		
Intron	Size(bp)	Location in cDNA
i	~650	bet.1064 & 1110
ii	~480	1240/1241
iii	~610	1499/1500
iv	~3000	1631/1632

Intron/Exon Boundaries		
Intron	Size(bp)	Location in cDNA
A	534	213/214
B	264	555/556
C	93	644/645
D	281	789/790
E	296	1433/1434
F	468	1619/1620
G	620	1830/1831

FIG. 6. Organization of the gene encoding r-*erbA*α-1, r-*erbA*α-2, and Rev-ErbAα. (A) Gene map of a region of the rat genome containing the r-*erbA*α and Rev-ErbAα genes. Both DNA strands are represented. Exonic sequences are depicted by open boxes, with the exception of the 269-nucleotide sequence, which is exonic in both orientations and is shaded black as in previous figures. Rev-ErbAα-exonic sequences are numbered. r-*erbA*α-exonic sequences are labeled α_c when common to r-*erbA*α-1 and r-*erbA*α-2. These exons are designated by lowercase letters. The r-*erbA*α-1-specific exon is labeled α-1; this region is intronic from the perspective of the r-*erbA*α-2 mRNA. Introns and intergenic sequences are indicated by lightly shaded boxes. Rev-ErbAα-introns are in capital letters. r-*erbA*α introns are designated by roman numerals. Intron *iv* is likely to contain some r-*erbA*α-1-exonic sequences which would represent additional 3′ untranslated nucleotides not present in the cDNAs which have been isolated. Similarly, the region of the Rev-ErbAα gene upstream of exon 1 is likely to contain an exonic sequence corresponding to the 5′ end of the mRNA, which is not present in the Rev-ErbAα cDNA. Arrows refer to the orientation corresponding to the indicated gene. Brackets highlight the exons containing the bidirectionally transcribed sequence. (B) r-*erbA*α-1, r-*erbA*α-2, and Rev-ErbAα cDNAs. Portions of the cDNAs corresponding to exons in Fig. 6A are indicated. Dots in front of the r-*erbA*α exons indicate that the 5′-most (~1,000-bp) region common to r-*erbA*α-1 and r-*erbA*α-2 is upstream of the genomic region analyzed and that the exonic composition of that region of the gene is not known. (C) Exon/intron boundaries in the r-*erbA*α and Rev-ErbAα genes. Introns in the Rev-ErbAα gene have been completely sequenced (data not shown); hence the sizes are exact. Sizes of introns in the r-*erbA*α gene have been estimated from partial sequencing, restriction enzyme analysis, and Southern blot hybridization studies (data not shown). Intron i is listed as a minimum of 600 bp in size because it extends beyond the *Eco*RI site to a region not yet characterized. Locations of introns in the r-*erbA*α cDNA refer to the r-*erbA*α-2 cDNA that we have previously described (25). Intron iv in the r-*erbA*α-2 splice product is composed of the r-*erbA*α-1-specific exon (α-1) in addition to the region shaded in Fig. 6A. Locations of introns in the Rev-ErbAα cDNA refer to Fig. 1B.

ligand-responsive DNA-binding transcriptional activators. Although the deduced Rev-ErbAα amino acid sequence is most similar to those of the thyroid hormone and retinoic acid receptors, the protein synthesized in vitro did not bind either T_3 or retinoic acid. This is not surprising in view of the fact that the similarity of Rev-ErbAα to either thyroid hormone receptors or retinoic acid receptors is no greater than the similarity of these receptors to each other. A number of "ligands in search of receptors" have been proposed, including dioxin, retinol, and cholesterol (9, 36). The fact that the Rev-ErbAα mRNA is most abundant in skeletal muscle and brown fat suggests interaction with a ligand involved in energy metabolism. We are currently searching for a ligand for Rev-ErbAα.

The DNA-binding region of Rev-ErbAα is similar to the homologous regions of the thyroid hormone and retinoic acid receptors but, again, as different from them as they are from each other. Thus, the different amino acids present in the DNA-binding region of the Rev-ErbAα protein, which occur at residues which tend to vary among members of the superfamily (Fig. 2A), suggest that Rev-ErbAα will interact with a different spectrum of genes than other members of the receptor superfamily.

The observation that the genes encoding Rev-ErbAα and the r-*erbA*α proteins are physically linked raises the question of whether the function of the Rev-ErbAα protein is related in any way to the r-*erbA*α-2 protein. Because of the structure of the genes, part of the carboxy terminus of the Rev-ErbAα protein is derived from the anticodons of a portion of the r-*erbA*α-2 gene encoding the carboxy terminus of the r-*erbA*α-2 protein. It has been suggested that amino acid sequences bearing this relationship may bind to one another (5).

Some tissues contain both the Rev-ErbAα and r-*erbA*α-2 mRNAs. If both mRNAs are expressed in the same individual cells, then their complementary nature could result in inhibition of translation of one or both (29). Sequences complementary at the 3′ ends, as in the present case, have been shown to be effective inhibitors of translation when generated in the nucleus (21).

Since r-*erbA*α-1 and r-*erbA*α-2 are derived from a single gene transcript, it is possible that the presence of the Rev-ErbAα mRNA, which is complementary to r-*erbA*α-2 but not r-*erbA*α-1, affects splicing of the r-*erbA*α-1/r-*erbA*α-2 primary transcript. Alternatively, transcription of the Rev-ErbAα gene might sterically hinder simultaneous transcription of the r-*erbA*α-2-specific exon, resulting in premature termination of the nascent r-*erbA*α gene transcript. In either case, the production of r-*erbA*α-1 mRNA might be increased relative to that of r-*erbA*α-2 mRNA. This is consistent with the fact that r-*erbA*α-1 mRNA is most abundant in brown fat and skeletal muscle (25), as is Rev-ErbAα mRNA.

The structural relationship between the Rev-ErbAα and r-*erbA*α genes may have evolved by duplication and inversion of a single precursor gene. This would be consistent with the observation that an intron is present between the exons encoding the zinc fingers in Rev-ErbAα at precisely the same sites as that in the chick c-*erbA*α gene (43) but not in the chick progesterone receptor (19) or human mineralcorticoid receptor (2) genes. In the course of evolution, as one of the duplicated genes diverged into the present Rev-

ErbAα sequence, its 3′-most exon may have acquired a different function, perhaps encoding a domain that binds a ligand other than T_3. The observed relationship between the r-*erbA*α and Rev-ErbAα genes could be explained by hypothesizing that the opposite strand of the 3′-most exon in the Rev-ErbAα gene became part of the r-*erbA*α gene transcript, and splicing of the r-*erbA*α transcript to yield the r-*erbA*α-2 mRNA in addition to r-*erbA*α-1 provided some selective advantage.

The Rev-ErbAα/r-*erbA*α genomic locus represents the first example of structurally related proteins encoded by overlapping transcriptional units in eucaryotes. Indeed, few other examples of bidirectional transcription have been discovered thus far. Most similar to the present case are the bidirectional transcription of the 3′-most exon of the dopa decarboxylase gene in *D. melanogaster* (39) and of the 3′-most exon of a mouse transcriptional unit of unknown function (42). In both of these cases, the significance of the opposite-strand transcription is unknown. Regulatory roles for the cRNAs have been postulated. A pupal cuticle protein gene is contained within intronic sequences of a *D. melanogaster* gene encoding three purine pathway enzyme activities (17). In this case, the functions of the proteins encoded by the opposite-strand transcripts are known, but no exonic sequences are present in both orientations in mature mRNAs. The rat gonadotropin-releasing hormone gene overlaps an opposite-strand transcript of unknown function (1). Transcription of the opposite strand of the c-*myc* gene has been presumed to serve a regulatory function, and mRNAs corresponding to the antisense transcripts have not been detected (22, 34). The discovery of additional examples of bidirectional gene transcription and further study of the examples already known will be necessary to better understand the prevalence and function of these potentially important relationships between certain genes.

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